even when DNA-binding is impaired, which suggests that the organization of mammalian nuclei plays a role in search dynamics. Next, we directly quantified the association rate constant of proteins to their targets in living cells. Based on our binding assay, the measured rate constant resulted much slower than expected and local decondensation of chromatin, at the target site locus, did not affect the association rate. In other words, the effective size of a DNA binding site is much smaller than the size of the molecular interaction.
The picture emerging from our experiments shows that, in human cells, specific DNA-binding proteins move in a complex environment where they may remain locally confined. They interact transiently also with nonspecific DNA sequences and their target search mechanism is consistent with a non-compact exploration of the nuclear space with very inefficient binding.

Platform: Ligand-gated Channels

2782-Plat
Incorporation of Unnatural Amino Acids into Trimeric Ion Channels
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The incorporation of unnatural amino acid side chains into ion channels has greatly aided the understanding of these physiologically and pharmacologically important membrane proteins. Although the so-called in vivo nonsense suppression method for the incorporation of unnatural amino acids is extremely versatile, not all ion channels appear to be amenable to this experimental approach. In fact, the technique has thus far been primarily applied to pentameric ligand-gated ion channels, and those in the voltage-gated channel family. Here, we show that unnatural derivatives of Phe and Trp can be successfully incorporated into the trimeric ASIC1 cation channel, from the ENaC/degenerin family of ion channels, as well as the P2X2 receptor, an ATP-gated ion channel. Importantly, unnatural derivatives were well tolerated at different positions in both extracellular and transmembrane domains, with rescued channels displaying robust expression and WT-like gating characteristics. The results suggest that these important ion channel families are amenable to novel chemical biology tools such as the incorporation of unnatural amino acids and thus open up new avenues for their study.

2783-Plat
Photoinactivation of Glutamate Receptors using a Genetically Encoded Unnatural Amino Acid
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Site-specific incorporation of unnatural amino acids has wide-ranging applications in the structure-function studies of proteins, offering high specificity, arbitrary environmental sensitivity of incorporation and a nearly unlimited variety of unnatural side chains. We have used photo-activatable unnatural amino acids to probe the activation and desensitization of glutamate receptors (GluRs), ligand-gated ion channels that mediate fast excitatory synaptic transmission in the brain. We introduced the unnatural amino acid cross-linker p-Benzoyl-Phenylalanine (Bpa) to the AMPA receptor GluA2 expressed in mammalian cell lines. Sites of interest were replaced by an amber stop codon (TAG). The stop codon was suppressed using a genetically-encoded exogenous tRNA and tRNA-synthetase pair that incorporate Bpa (Ye et al, 2008 J Biol Chem). We initially chose sites in the lower (D2) lobes of the LBD (S729, G725), which have been previously shown to form intermolecular disulfide cross-links that inhibit the receptor (Armstrong et al, 2006 Cell; Plested and Mayer, 2009 J. Neurosci). Patch clamp electrophysiology coupled to rapid perfusion demonstrated that Bpa-rescued GluRs had similar properties to wild-type GluRs. Receptors could be trapped in inactive conformational states upon UV irradiation and subsequent photo-cross-linking, consistent with previous work. Irreversible trapping was characterized by near-complete peak current reduction (95 %) in less than five seconds cumulative exposure to UV at 365 nm from a mercury lamp. The cross-linking rate depended on UV intensity and exposure time. Wild-type channels were unaffected by similar UV exposures. Our results demonstrate that genetically-encoded unnatural amino acids such as Bpa provide an convenient strategy to control structural rearrangements of ligand-gated ion channels in mammalian cells.

2784-Plat
Conformational Transitions Underlying Pore Opening and Desensitization in Membrane-Embedded GLIC
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Direct structural insight into the mechanisms underlying activation and desensitization remain unavailable for the pentameric ligand-gated channel family. Here, we report the structural rearrangements underlying gating transitions in membrane-embedded GLIC, a prokaryotic homologue, using site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy. We particularly probed the conformation of pore-lining second transmembrane segment (M2) under conditions that favor the closed and the ligand-bound desensitized states. The spin-label mobility, intersubunit spin-spin proximity and the solvent-accessibility parameters in the two states clearly delineate the underlying protein motions within M2. Our results show that during activation the extracellular hydrophobic region undergoes major changes involving an outward translational movement, away from the pore axis, leading to an increase in the pore diameter, while the lower end of M2 remains relatively immobile. Most notably, during desensitization, the intervening polar residues in the middle of M2 move closer to form a solvent-occluded barrier and thereby reveal the location of a distinct desensitization gate. In comparison to the crystal structure of GLIC, the structural dynamics of the channel in a membrane environment suggest a more loosely packed conformation with water-accessible intrasubunit vestibules penetrating from the extracellular end all the way to the middle of M2 in the closed-state. These regions have been implicated to play a major role in alcohol and drug modulation. Overall, these findings represent a key step towards understanding the fundamentals of gating mechanisms in this class of channels.

2785-Plat
Proton Binding and Conformational Dynamics in KcsA
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Though KcsA has been the subject of extensive studies, the specific molecular mechanism by which the channel opens in response to proton binding is unknown. We used a multi-pronged approach - electrophysiology, X-ray crystallography, modeling, and NMR - to understand pH-dependent gating in KcsA. We propose that the pH sensor consists of a strong sensor, H25, in addition to one weak sensor, E118. Protonation of these residues drives opening of the channel by shifting the equilibrium from the closed state to the open state. We modeled KcsA pH gating using a modified Monod-Wyman-Changeux (MWC) model with 2 proton binding sites per subunit. In this model, the pKa values for each pH sensor are different in the closed state than in the open state, as required for proton binding to drive opening of the channel. This model choice is reasonable as the conformational changes required to open the channel do produce an alternate environment for H25 and E118, effectively altering their pKa values. We sought to constrain this model by determining the pKa values for these residues directly using solution NMR. using KcsA reconstituted into bicelles, we determined that H25, the strong sensor, responds to pH changes in two distinct conformational states, with an apparent pKa in the range of channel gating. Additional NMR studies targeted at the weak proton sensor, E118, and other bundle-crossing mutants will provide further insight into the coupling of proton binding with channel gating.

2786-Plat
Reversible Gating of the NaK Channel by Changes in Redox Potential
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NaK is a two transmembrane, monovalent cation channel from B. cereus. The crystal structures of NaK wt (Shi et al Nature 2006) and the deletion construct NaK Δ1-19 (Alam et al NSMB 2009) are thought to represent the closed and open states of the channel, respectively. Yet, while this has been an area of intense research, NaK's physiological mechanism of activation remains to be established. Indeed, although a marginal ionic flux can be detected in liposome-reconstituted wt NaK, neither wt nor Δ1-19 show significant ionic current unless additional gain-of-function mutations are introduced: F92A in the Δ1-19 deletion (Derebe et al PNAS 2010) or F94L in wt (Bourdeau et al BPS Mtg. 2011 3159-Pos). Though useful, these mutations limit most functional measurements to stationary analysis, which cannot differentiate activation from inactivation gating events. Here, we present evidence suggesting that NaK is a redox-gated channel. Under oxidizing conditions the channel has a single-channel conductance of 75 pS with an open probability of 0.65, as measured by patch-clamp electrophysiology on reconstituted proteoliposomes. Upon addition of DTT, the channel reversibly closes, opening the possibility of carrying out non-stationary analysis of the channel. Although the nature of the redox sensor remains to be established, these results strongly suggest that cellular redox potential might be the defining physiological activator of NaK.

2787-Plat
Dimeric TRIP8b Binds to the Cyclic Nucleotide Binding Domain of HCN Channels
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